OVA Specific IgE ELISA

Coat plates with 50ul of 4ug/ml OVA in carbonate buffer o/n @4°C.

Dilute samples at appropriate dilution (1:50, 1:200, 1:800, 1:3200).

Wash plate and add 50 ul of blocking buffer and 50 ul of diluted sample. Let stand on a rotor @ rt for 3+ hrs.

Wash plate and add 100 ul anti mouse IgE-HRP (1:1000). Let stand on a rotor @ Rt for 1 hr.

Add O PD (0.25 mg/ml) and UPO (0.2 mg/ml) in citrate phosphate buffer.

Read OD at 492nm.

Use the OD of one sample to base all others on. Consider the first dilution neat, 1:4, 1:16 and 1:64 and construct curve OD vs dilution.

Take the half max of the OD and figure the dilution necessary to achieve that dilution, take it as 1 unit. Replot graph with unit vs OD. Multiply units for each sample by dilution factor x 10 to get the final unit/ml.

$$40 \, mg / 50 ml = 0.8 \, mg/ml$$

$$\frac{4 \, mg/ml}{800 \mu g/ml} \times 50 = 250 \mu l. + 50 \mu l.$$

120 µl

2 plates

1   2   3   4   5   6   7

b| OO 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 8

G| OO   G-3 8

µl IgE

H| OO   ⁰A 3

B| OO   ⁰B 3

E| OO   ⁰C 3

µl IgE

Control	Group G	Group D	Group E
25.82	121.38	126.72	36.3
13.1	71.46	164.8	54.5
32.76	102.46	201.56	42.14
41.7	107.84	172.57	54.4
28.345	100.785	166.4125	46.835
12.0641881	21.1083577	30.829403	9.11062932
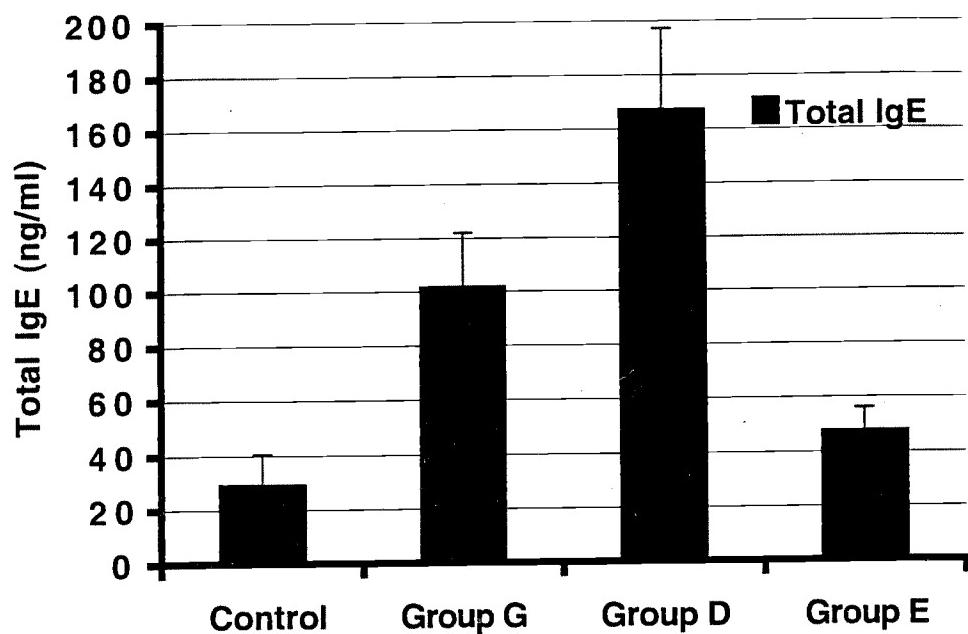
Control	Group A	Group B	Group C
16.26	139.45	400	54.4
11.495	109.56	360	46.7
32.84	119.76	370	50.94
37.2	145.12	439	63.4
24.44875	128.4725	392.25	53.86
12.488164	16.6456709	35.5	7.09683498
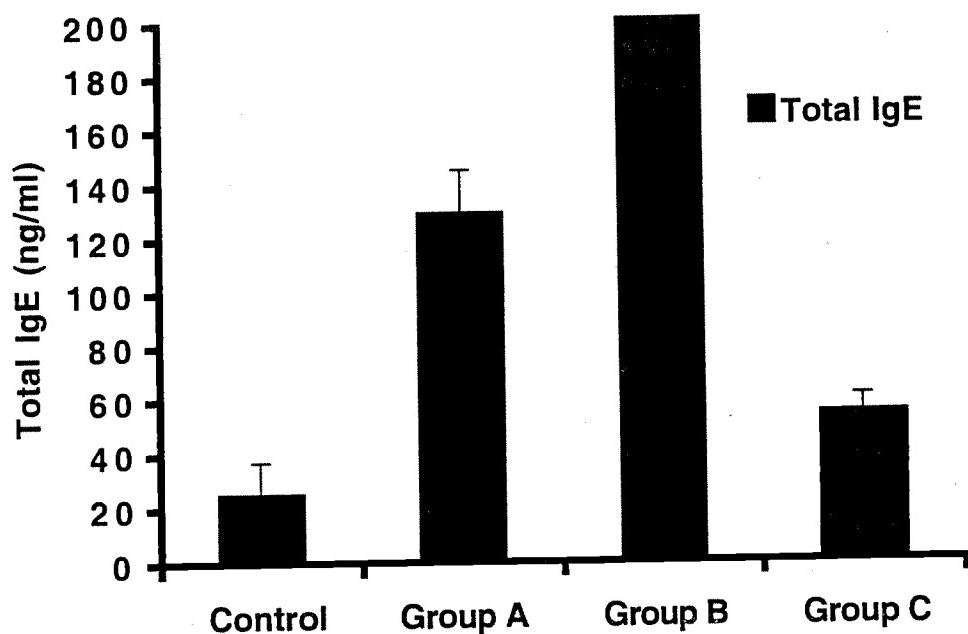
	Medium	OVA	OVA+RM134L	RM134L
OVA-Alum	1918	6883	6087	1947
Alum	1700	2787	3899	1346
OVA-Alum-RM134L	1518	3211	5353	2100
	386	1742	1674	471
	524	509	818	426
	239	1394	1379	454

72 hrs Spleen proliferation assay
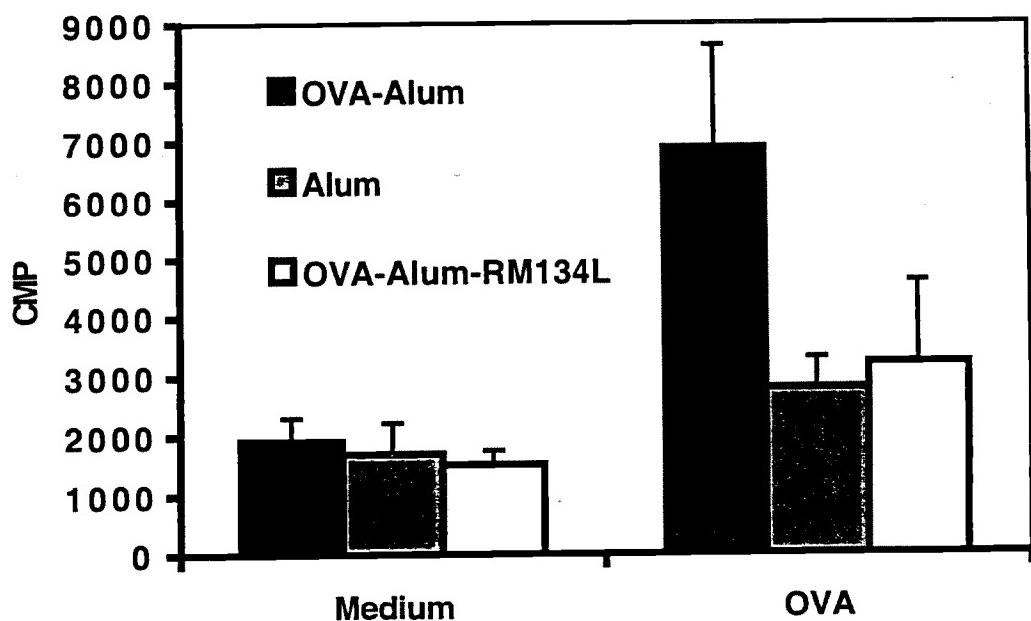
	Medium	OVA	OVA+RM134L	RM134L
OVA-Alum	2781	6593	5389	3210
Alum	2718	3364.7	3709	3156
OVA-Alum-RM134L	1541	2891	1760	863
	111	253	1247	308
	523	429	139	152
	361	539	464	224

72 hrs LUNG proliferation assay